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Rotifers Ingest Oocysts of *Cryptosporidium parvum*

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ABSTRACT. Six genera of rotifers including *Philodina, Monostyla, Epiphanes, Euchlanis, Brachionus,* and *Asplanchna* were exposed to oocysts of *Cryptosporidium parvum* cleaned of fecal debris. Unstained oocysts and those stained with fluorescein-conjugated monoclonal antibody were added to suspensions of viable rotifers and were examined by phase-contrast, differential interference contrast, and fluorescence microscopy. Rotifers of all six genera were observed ingesting oocysts. A maximum of 25 oocysts was observed in the stomachs of *Euchlanis* and *Brachionus. Euchlanis* and *Epiphanes* were observed excreting boluses containing up to eight oocysts. It was not determined whether rotifers digested or otherwise rendered oocysts nonviable.

Key Words. Behavior, cryptosporidiosis, protozoa, sewage, waterborne, zoonosis.

THE Class Monogononta comprises the largest group of rotifers with more than 2,000 species in about 95 genera of benthic, free-swimming, and sessile forms. Most are found in freshwater lakes, ponds, puddles, damp soil, moss, or any place where water can accumulate, such as bird baths, in brackish water, and to a lesser extent in salt water (Nogrady, Wallace, and Snell 1993). They thrive in highly eutrophic environments such as domestic sewage and waste water where their predators, copepods, cannot survive for very long. Rotifers range from 100–1,000 μm in length. They possess an anteriorly located ciliated corona used in locomotion and food gathering (Meglitsch 1972). The structure varies widely among species but cilia sweep water and suspended particles towards a centrally located oral cavity where an inner set of cilia reject any unsuitable food (Meglitch 1972: Gilbert and Starkweather 1997, 1998). Particles swept into the oral cavity pass into the mastax, a muscular organ with plates that chop or push particles into the esophagus, the stomach, and then into an intestinal tube (Nogrady, Wallace, and Snell 1993). Five digestive glands have been reported for one rotifer species (Schramm 1978) and digestive enzymes, endoglucanases and glycosidases, have been identified in homogenates from another species (Chun, Hur, and Kim 1997; Kuhle and Kleinow 1990).

Cryptosporidiosis, a diarrheic disease of humans, livestock, and wild animals, results after the oocyst stage of *Cryptosporidium parvum* is excreted in the feces of an infected mammal and ingested by a susceptible host of the same or another species (Fayer, Speer, and Dubey 1997). Oocysts of *C. parvum* are widely distributed in surface waters and *C. parvum* has become the most important newly recognized contaminant in drinking water in the United States (Rose, Lisle, and LeChevalier 1997). Rivers, lakes, springs, and groundwater have all been implicated as sources of contaminated water in five well-documented outbreaks of cryptosporidiosis from drinking water affecting from 500–400,000 persons (Rose, Lisle, and LeChevalier 1997). Because oocysts of *C. parvum* are within the size range of particles ingested by rotifers, the present study was conducted to determine if any freshwater rotifer species might ingest and possibly also digest waterborne oocysts. If so, some clues might emerge as to the fate of oocysts in the environment or might provide insight for removal of oocysts from water contaminated with human or animal feces.

MATERIALS AND METHODS

Oocysts of *C. parvum* were cleaned from the feces of experimentally infected calves and used within 1 mo. Briefly, the fecal material was passed through a series of sieves with increasingly finer mesh, subjected to continuous flow, differential density gradient centrifugation, and further purified by centrifugation on a discontinuous cesium chloride (CsCl) gradient as described (Kilani and Sekla 1987), and then oocysts were extensively washed to remove CsCl residue. Some purified oocysts were immediately resuspended in filtered spring water at a concentration of 2×10^7 oocysts ml^{-1}. Others were labeled with fluorescein-conjugated monoclonal antibody (Merifluor; Meridian Diagnostics, Cincinnati, OH) by resuspending 2×10^7 oocysts in 200 μl of detection reagent. After a 1-h incubation at room temperature, the oocysts were thoroughly washed by suspending them in 15 ml of 0.2 μm-filtered spring water, centrifuging at 1,500 *g* for 15 min, and decanting the supernatant. The oocysts were then resuspended in 1 ml of the same water, yielding a final concentration of nearly 2×10^7 *C. parvum* oocysts/ml.

The rotifers, *Philodina* sp. and *Monostyla* sp., were obtained from Carolina Biological Supply Company (Burlington, NC). *Epiphanes brachionus, Euchlanis triquetra, Brachionus quadridentatus,* and *Asplanchna* sp. were provided by Elizabeth Walsh of the Department of Biological Sciences, University of Texas at El Paso. Rotifers were maintained in 100-ml polyethylene screw-capped jars in filtered spring water and were fed approximately every two days with a mixture of *Ankistrodesmus falcatus* (UTEX90) and *Chlamydomonas reinhardti* (UTEX90). To select rotifers for testing, 30 μl of the culture were aspirated by pipette from a heavily populated area on the bottom of the jar, and 10 μl, containing approximately 10–20 rotifers, were placed into each of three 11-mm diameter wells of a heavy teflon-coated three-well slide (Erie Scientific, Portsmouth, NH). In less concentrated cultures, individual organisms, observed at 200× with an Olympus CK2 inverted microscope, were aspirated by pipette and placed onto slides. Approximately 48 h had passed since the last addition of food to the cultures although food organisms were still visible in all cultures. One μl of oocyst suspension (2×10^4 oocysts), either unlabeled or labeled with monoclonal antibody, was added to two of the wells. The third well served as an oocyst-free control. Slides were covered with 24 × 50-mm glass coverslips that were ringed with vaseline to prevent evaporation and flow of the suspension. Rotifers of all six genera were observed with fluorescence, phase-contrast, and differential interference contrast (DIC) microscopy, using a Zeiss Axioskop equipped with an FITC filter, a Texas Red/FITC dual wavelength filter, and DIC optics. Approximately 30 slides were prepared, examined, and photographed for six genera. Photomicrographs on Kodak Elite 100 color transparency film were made with a Zeiss autoexposure MC80 camera system. Color transparencies were scanned with a Polaroid Sprint Scan 35 and the digitized images were used to create black and white positive photographs using

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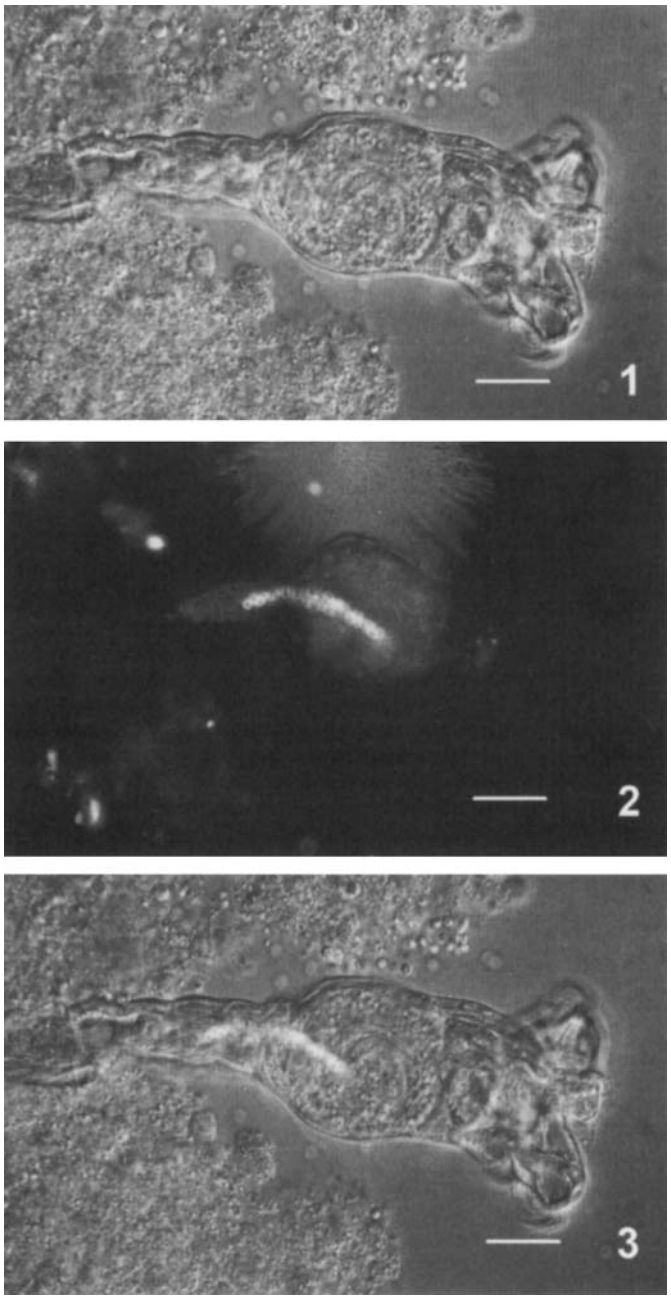

Fig. 1–3. *Philodina* containing approximately 25 ingested oocysts of *Cryptosporidium parvum*. Fig. 1. *Philodina* is clearly visible but ingested oocysts are not visible when observed by differential interference contract microscopy. Fig. 2. The same *Philodina* observed by fluorescence microscopy with a row of fluorescing oocysts clearly visible in the intestine of the poorly visible rotifer. Fig. 3. A composite of Fig. 1 and 2 merged by computer software. Both the rotifer and ingested oocysts are clearly visible. Bar = 40 μm.

Adobe Photoshop 5.0 software. This software was also used to create a composite image from individual fluorescence and DIC photomicrographs of the same rotifer taken sequentially (Fig. 1–3).

RESULTS AND DISCUSSION

This is the first report in which rotifers have been observed ingesting any stage of a protozoan parasite of vertebrates. Rotifers of several genera are reported to be parasitized with Microsporidia (Wallace and Snell 1991). In the present study, rotifers of six genera were observed in the living state and all were seen ingesting oocysts of *C. parvum*. However, quantitation within genera and between genera was variable because high magnification was required to identify the small oocysts (4.5–5.5 μm in diam.) while rotifers were highly mobile or active and relatively large, requiring two-dimensional microscope stage movement and continuous focusing through the body while both the rotifer and internal contents were in motion. Initial observations using unlabeled oocysts revealed that both *Philodina* and *Monostyla* ingested the oocysts, but oocysts within the bodies of these rotifers were difficult or impossible to visualize (Fig. 1). Therefore, subsequent observations with these and other genera were made using oocysts labeled with fluorescein-conjugated monoclonal antibody and examined with fluorescence microscopy. The influence of monoclonal labeling with regard to enhancing or diminishing oocyst ingestion is unknown.

Philodina sp., *E. triquetra,* and *B. quadridentatus* consistently ingested the greatest number of oocysts. The oocysts ingested by *Philodina* were visible in a long, slender, somewhat coiled tube, possibly the intestine, posterior to the jawlike mastax (Fig. 2, 3). Some *Philodina* contained as many as 25 oocysts, but many contained five to 15. In *E. triquetra* and *B. quadridentatus* (Fig. 4), oocysts were visible in the stomach. Within the stomach vigorous mixing of up to 25 oocysts and other material was observed, so the actual number of oocysts could not be accurately determined. Attempts to improve quantitation by examining fixed specimens were not fruitful because many rotifers lysed and/or expelled their contents on fixation. Oocysts were also seen within *Monostyla* sp., *E. brachionus,* and *Asplanchna* sp., but these rotifers consistently appeared to have ingested fewer oocysts than the other species. Many oocysts that were drawn to the corona by ciliary action appeared to be rejected. It is not known if oocysts were physically or otherwise unacceptible but many appeared to be momentarily ingested and then expelled.

Both *E. triquetra* and *E. brachionus* were observed excreting boluses of up to eight oocysts clumped within unidentified material. Oocysts appeared to fluoresce brightly and to retain a spherical shape. The time of microscopic observation from exposure of rotifers to oocysts until boluses were excreted by *E. triquetra* and *E. brachionus* was approximately 15 min. It was not determined during that time period if oocysts within the rotifers underwent any digestion, degradation or inactivation, but digestion in rotifers reportedly takes 15–20 min. (Kuhle and Kleinow 1990). Oocysts ingested by rotifers of other genera appeared to be retained internally throughout the period of observation. Small fluorescing granules that could represent fragments of oocyst wall or possibly dissociated fluorescein were observed in some rotifers. However, actual digestion with dissolution or destruction of the oocyst wall and contents could not be confirmed. Enzymes identified in rotifers digest primarily carbohydrate substrates (Chun, Hur, and Kim 1997; Kuhle and Kleinow 1990). It is not known if rotifers possess enzymes capable of digesting the proteins thought to make up the wall of oocysts.

Observations in the present study were conducted on rotifers artificially exposed to oocysts of *C. parvum*. Whether rotifers ingest oocysts in nature is unknown. The application of the present laboratory findings to field conditions such as farm ponds, sewage lagoons, wastewater treatment facilities or other locations where oocysts might enter the environment in large numbers should be attempted. Although it is unknown whether oocysts are degraded in any way after ingestion by rotifers, it

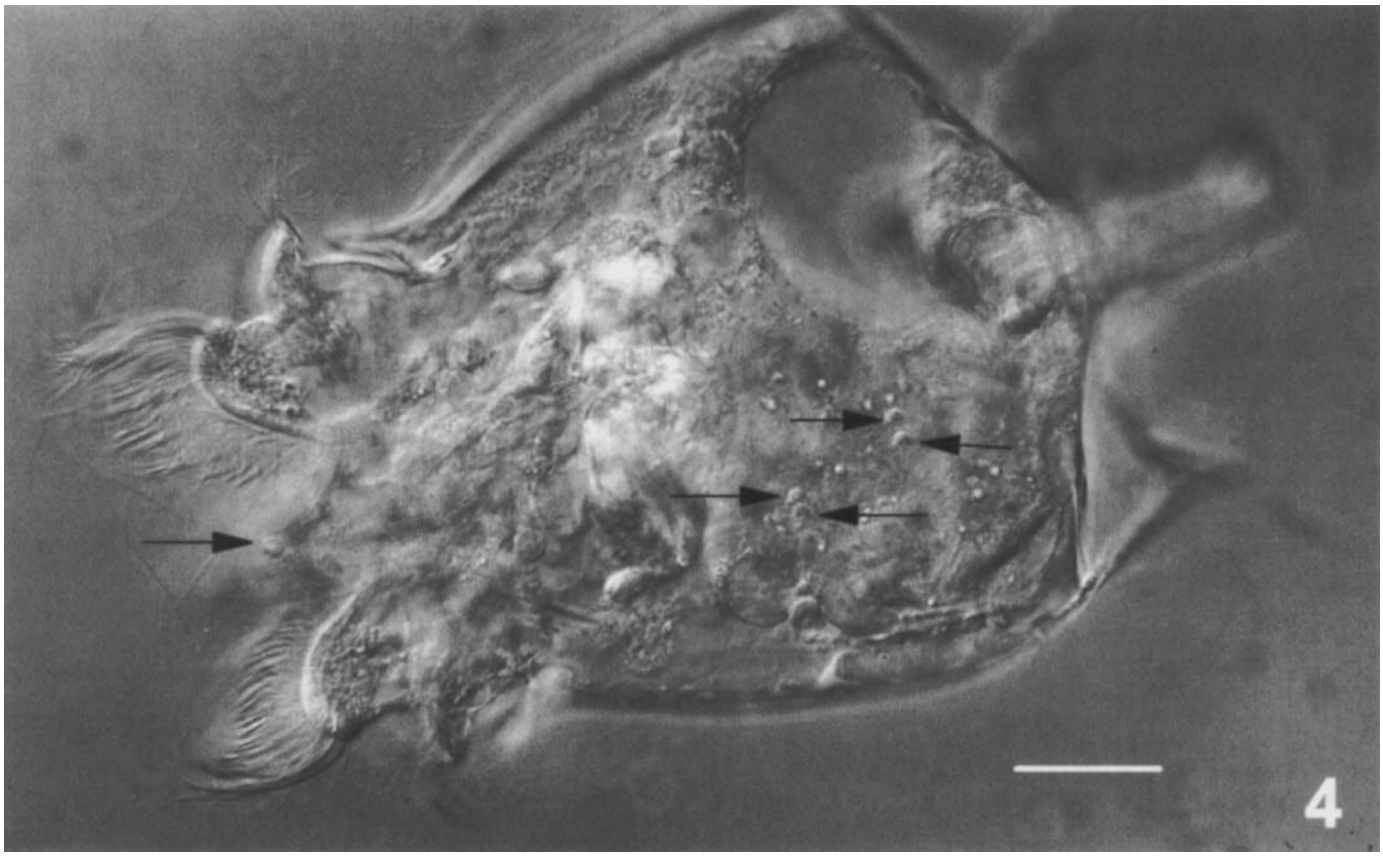

Fig. **4**. Photomicrograph of a live rotifer, *Brachionus quadridentatus,* observed by differential interference contrast microscopy, with four ingested *Cryptosporidium parvum* oocysts and one oocyst anterior to the ciliated corona (arrows). Oocysts were confirmed by fluorescence microscopy. Bar = 33 μm.

was observed that oocysts were excreted by some rotifers in boluses containing a mixture of other ingested materials. Even if these boluses, 20–30 times larger than individual oocysts, contained oocysts that remained infectious, their large size could facilitate removal from water by filtration.

LITERATURE CITED

Chun, C. Z., Hur, S. B. & Kim, Y. T. 1997. Purification and characterization of an endoglucanase from the marine rotifer *Brachionus plicatilis. Biochem. Mol. Biol. Int.,* **43**:241–249.

Fayer, R., Speer, C. A. & Dubey, J. P. 1997. The general biology of *Cryptosporidium. In*: Fayer, R. (ed.), *Cryptosporidium* and cryptosporidiosis. CRC Press, Boca Raton, p. 1–41.

Gilbert, J. J. & Starkweather, P. L. 1977. Feeding in the rotifer *Brachionus calicyflorus.* I. Regulatory mechanisms. *Oecologia (Berlin),* **28**:125–131.

Gilbert, J. J. & Starkweather, P. L. 1978. Feeding in the rotifer *Brachionus calicyflorus.* III. Direct observations on the effects of food type, food density, change in food type, and starvation on the incidence of pseudotrochal screening. *Int. Vereinig. theoret. ange. Limnol., Verhandl.,* **20**:2382–2388.

Kilani, R. T. & Sekla, L. 1987. Purification of *Cryptosporidium* oocysts and sporozoites by cesium chloride and percoll gradients. *Am. J. Trop. Med. Hyg.,* **36**:505–508.

Kuhle, K. & Kleinow, W. 1990. Glycosidases in *Brachionus plicatilis* (Rotifera). *Comp. Biochem. Physiol.,* [B], **95**:393–402.

Meglitsch, P. A. 1972. Invertebrate Zoology, 2d ed. Oxford University Press, New York, p. 216–226.

Nogrady, T., Wallace, R. L. & Snell, T. W. 1993. Rotifera. Vol. 1: Biology, ecology and systematics. *In*: Dumont, H. J. F. (ed.), Guides to the Identification of the Microinvertebrates of the Continental Waters of the World. SPB Academic Publishing, The Hague, The Netherlands.

Rose, J. B., Lisle, J. T. & LeChevallier, M. 1997. Waterborne cryptosporidiosis: incidence, outbreaks and treatment strategies. *In*: Fayer, R. (ed.), *Cryptosporidium* and Cryptosporidiosis. CRC Press, Boca Raton. p. 93–109.

Schramm, U. 1978. Studies of the ultrastructure of the rotifer *Habrotrocha rosa* Donner (Aschelminthes). The alimentary tract. *Cell Tiss. Res.,* **189**:525–535.

Wallace, R. L. and Snell, T.W. 1991. Rotifera, *In*: Thorp, J. H. & Covish, A. P. (ed.), Ecology and Classification of North American Freshwater Invertebrates. Academic Press, Inc., New York. p.187–248.

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